Prostaglandin D Synthase (β-Trace) in Meningeal Hemangiopericytoma

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The level of prostaglandin D synthase (PGDS), a major protein constituent of cerebrospinal fluid (CSF), is altered in various brain diseases, including meningitis. However, its role in the brain remains unclear. PGDS is mainly synthesized in the arachnoid cells, the choroid plexus and oligodendrocytes in the central nervous system. Among brain tumors, meningiomas showed intense immunoreactivity to PGDS in the perinuclear region. Thus, PGDS has been considered a specific cell marker of meningioma. In this study, we examined 25 meningeal hemangiopericytomas (HPCs) and found that 16 of the tumors (64%) showed immunoreactivity for PGDS in the perinuclear region. For comparison, 15 meningiomas, 14 soft-tissue HPCs, 1 mesenchymal chondrosarcoma, 3 choroid plexus papillomas, and 7 oligodendrogliomas were also examined. Meningiomas showed positive immunoreactivity for PGDS in 13 cases (80%). Except for one case located at the sacrum, none of the other soft-tissue HPCs showed immunostaining for PGDS. Mesenchymal chondrosarcoma arises in the bones of the skull, and its histological pattern resembles that of HPC; however, it showed no immunoreactivity for PGDS. Neither choroid plexus papillomas nor oligodendrogliomas were immunopositive for PGDS. These findings suggest that meningeal HPCs may have a unique molecular phenotype that is distinct from that of the soft-tissue HPCs. The origin of meningeal HPCs may be more closely related to the arachnoid cells.

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Meningeal hemangiopericytoma (HPC), formerly regarded as a variant of angioblastic meningioma, represents an uncommon type of perivascular soft-tissue tumor (1). In 1942, Stout and Murray (2) identified a soft-tissue tumor located primarily in the thigh, buttock, and retroperitoneum that seemed to consist of proliferating pericytes and called it a *hemangiopericytoma.* Begg and Garrett (3) first reported a meningeal HPC in 1954. They reviewed six angioblastic meningiomas from Cushing's series and concluded that they were actually HPCs arising from within the meninges. Today, most pathologists are convinced that meningeal HPC and soft-tissue HPC are similar (4). Therefore, meningeal HPC is thought to be a pericytic tumor and is classified as a different entity from meningioma.

Prostaglandin D synthase (PGDS) [prostaglandin H_2 D isomerase; (5Z,13E)-(15S)-9,11-epidioxy-15-hydroxyprosta-5,13-dienoate-D-isomerase; EC 5.3.99.2] is a brain-specific glycoprotein that regulates sleep through the synthesis of PGD_2. PGDS is a member of the lipocalin superfamily composed of various secretory lipophilic ligand-carrier proteins (5–7). PGDS and its mRNA are mainly synthesized in the arachnoid cells, the choroid plexus, and oligodendrocytes in the central nervous system (8–10). In brain tumors, only meningioma cells have been proved to show intense immunoreactivity to PGDS in the perinuclear region (10). PGDS has thus been considered a specific cell marker of meningioma. The present study presents an immunohistochemical comparison of 25 meningeal HPCs, 15 meningiomas, 14 soft-tissue HPCs, 1 mesenchymal chondrosarcoma, 3 choroid plexus papillomas (CPPs), and 8 oligodendrogliomas with respect to the brain-specific protein PGDS.

MATERIALS AND METHODS

Cases

The tumor samples listed in Table 1 were obtained at surgery from 23 patients (17 male and 6 female, including two recurrent cases; ranging in age from 13–66 y) at the Department of Neurosurgery, Kyushu University Hospital. The tumor samples listed in Tables 2–4 were also submitted at surgery from 14 patients in Table 2 (8 male and 6 female; ranging in age from 0–65 y), 1 patient in Table 3 (male; 12 years old) and 15 patients in Table 4 (4 male and 11 female; ranging in age from 41–83 y) at the Departments of Surgery, Orthopedics, and Neurosurgery, Kyushu University Hospital. Three CPPs (from one male and two female patients ranging in age from 17–67 y) and seven oligodendrogliomas (from six male and one female patients ranging in age from 31–69 y) were also obtained at the Department of Neurosurgery, Kyushu University Hospital. To assure optimal immunoreactivities, only those tumors resected after 1975 were included in the study.

Immunohistochemistry

Immunohistochemistry for PGDS was performed on paraffin sections of brain tumors and soft-tissue HPCs by the indirect immunoperoxidase method.

Surgical specimens of the tumors were fixed in 10% buffered formalin overnight and embedded in paraffin. The samples were then cut into 5-μm sections. The sections were deparaffinized in xylene and hydrated in an ethanol gradient. The endogenous peroxidase activity was blocked with 0.3% H_2O_2 in absolute methanol for 30 minutes at room temperature. The sections were then washed in TB (50 mM Tris-HCl, pH 7.6), followed by overnight incubation with the PGDS antibody (1:2000 dilution, kindly supplied by Mr. Oda, Central Research Institute, Maruha Corporation, Tsukuba, Japan) at 4°C. After being washed in TB, the sections were incubated with horseradish peroxidase-conjugated secondary antibody (1:200 dilution, Vector Laboratories, Burlingame, CA). The colored reaction product was developed with 3,3'-diaminobenzidine tetrahydrochloride (3,3'-diaminobenzidine) solution. The sections were counterstained lightly with hematoxylin. The tests were done together with an appropriate positive control (meningioma).

TABLE 1. PGDS Immunoreactivity in Meningeal Hemangiopericytomas

Location	Number of Cases	PGDS Immunopositivity (%)
Supratentorial	18	14 (78)
Infratentorial	3	1 (33)
Thoracic spine	4	1 (25)
Total	25	16 (64)

PGDS, prostaglandin D synthase.

TABLE 2. PGDS Immunoreactivity in Soft-Tissue Hemangiopericytomas

Location	Number of Cases	PGDS Immunopositivity (%)
Retroperitoneum	4	0
Femur	2	0
Buttock	2	0
Other regions	6	1
Total	14	1 (7)

PGDS, prostaglandin D synthase.

TABLE 4. PGDS Immunoreactivity in Meningiomas

Subtype	Number of Cases	PGDS Immunopositivity (%)
Meningothelial	7	7
Fibrous	3	1
Transitional	3	3
Secretory	2	1
Total	15	12 (80)

PGDS, prostaglandin D synthase.

TABLE 3. PGDS Immunoreactivity in Mesenchymal Chondrosarcoma

Location	Number of Cases	PGDS Immunopositivity
Supratentorial	1	0

PGDS, prostaglandin D synthase.

Immunoblot Analysis

The specificity of the polyclonal antibody against PGDS in tumor tissue was assessed using a soluble fraction extracted from frozen tumor samples of meningeal HPCs and meningiomas. The samples were homogenized in 1.5 volumes of buffer containing 2% sodium dodecyl sulfate, 2 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl, pH 6.8. The protein concentrations were determined by a modified Lowry's procedure using bovine serum albumin as the protein standard. Laemmli's sample buffer was added to this mixture, and the samples were boiled for 5 minutes. Each protein sample (15 μg per lane) was separated on 12% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, Massachusetts). After blocking with 5% low-fat milk in TBST (25 mM Tris-HCl, pH 7.6; 0.15 M NaCl; 0.05% Tween 20; 0.05% NaN_3), the membrane was incubated at 4°C overnight with anti-PGDS antibody (1:2000) in TBST containing 5% low-fat milk. After it was washed, the filter was incubated with the alkaline phosphatase-conjugated secondary antibody (1:7500 dilution, Promega, Madison, WI), and the blot was visualized by the substrates of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

RESULTS

Clinical Information

Of the 25 meningeal HPCs, 18 (72%) were supratentorial, commonly parasagittal or falcial; 3 (12%) were infratentorial, one each located in the cerebello-pontine angle, jugular foramen, and torcular Herophili; and 4 (16%) were in the thoracic region (Table 1). No purely intraparenchymal HPC was encountered. Fourteen soft-tissue HPCs were located in a variety of somatic regions, including four in the retroperitoneum; two in the femur; two in the buttock; and a single case each from the breast, shoulder, and anterior sacrum (Table 2). The single case of mesenchymal chondrosarcoma was located in the parietal region (Table 3). Fifteen cases of meningioma consisted of four subtypes, including seven meningothelial, three fibrous, three transitional, and two secretory (Table 4). Among the meningiomas, 12 cases were supratentorial.

Pathology

Meningeal and soft-tissue HPCs were typical cellular tumors composed of oval to slightly spindle cells with oval, occasionally elongated nuclei (Fig. 1A). Nuclear atypia and mitosis were seen but varied from case to case. The tumor cells grew as monotonous sheets, interrupted by numerous slit-like vascular spaces lined by flattened endothelial cells. So-called staghorn sinusoids were identified in all cases. Mesenchymal chondrosarcoma showed a biphasic pattern of well-differentiated cartilage alternating with cellular portions resembling an HPC. Meningiomas had a wide range of histopathological appearances characteristic of the subtypes.

Immunohistochemistry

The results of our immunohistochemical analysis for PGDS are summarized in Tables 1–4. Meningeal HPCs were immunopositive for PGDS in 64% of all cases examined (Table 1). Especially those in the supratentorial region showed more frequent immunoreactivity (78%) than did those in either the infratentorial regions (33%) or the thoracic cord (25%). Staining tended to be focal and patchy compared with meningioma; however, perinuclear, granular cytoplasmic staining resembling meningioma was observed (Fig. 1B). PGDS expression was observed in neither soft-tissue HPC (Table 2, Fig. 1C) nor mesenchymal chondrosarcoma (Table 3), except for a single case of soft-tissue HPC located at the sacrum (Fig. 1D). Meningioma showed higher frequency (80%) of immunostaining for PGDS (Table 4). Typical staining was observed around the perinuclei (Fig. 2, A–B). Two cases of fibrous meningioma and one case of secretory meningioma showed no immunoreactivity for PGDS. Neither CPPs nor oligodendrogliomas were immunopositive for PGDS.

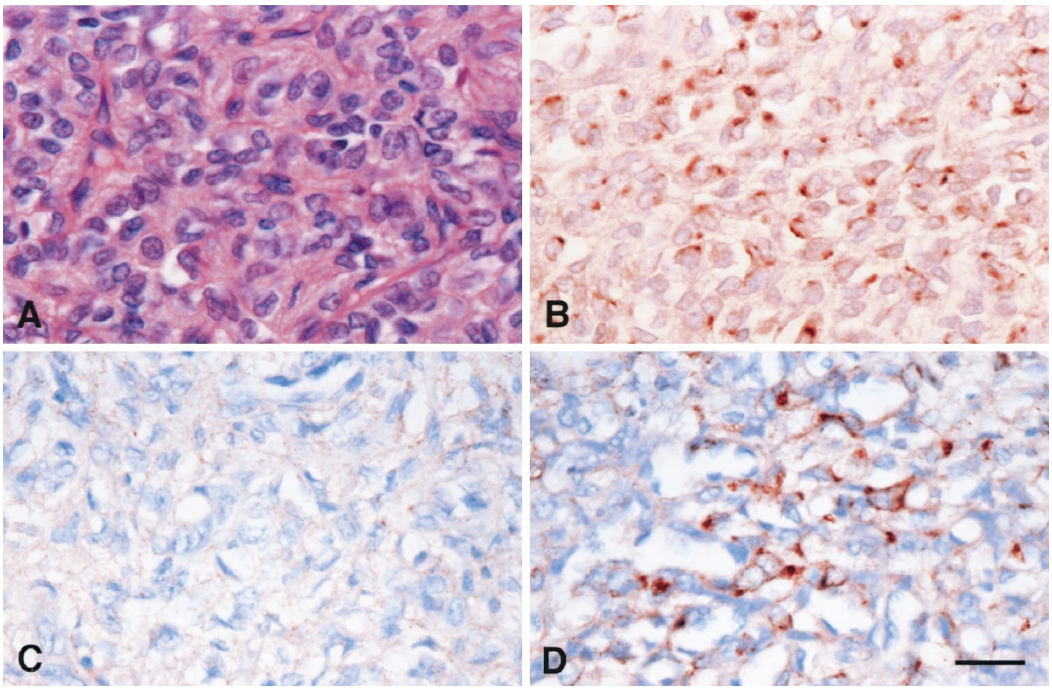

FIGURE 1. Hemangiopericytomas of both meninges and soft tissue. **A**, meningeal hemangiopericytoma (HPC) is a cellular and vascular tumor composed of round to oval cells with oval nuclei that is indistinguishable from soft-tissue HPC (**HE**). **B**, immunohistochemical staining for prostaglandin D synthase (PGDS) in meningeal HPC reveals perinuclear, cytoplasmic immunoreactivity. **C**, soft-tissue HPC. Immunoreactivity for PGDS is not observed. **D**, soft-tissue HPC of the sacrum. The tumor shows cytoplasmic staining for PGDS resembling meningeal HPC. (bar = 50 μm)

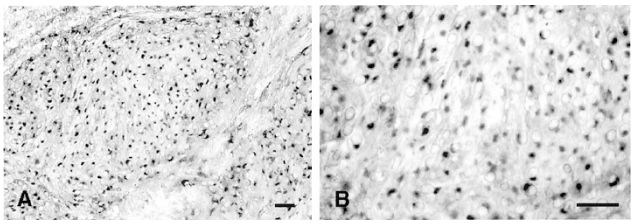

FIGURE 2. Meningothelial meningioma immunostained for prostaglandin D synthase (PGDS). **A** and **B**, the tumor shows perinuclear, cytoplasmic staining for PGDS similar to the positive pattern in the meningeal hemangiopericytoma. (bar = 50 μm)

Immunoblot Analysis

The results of immunoblotting are shown in Figure 3. The extracts from meningeal HPCs (Lanes 1 and 2) showed a band (29 kDa) corresponding to PGDS. Meningiomas (Lanes 3–5) also showed a same–molecular-weight band.

DISCUSSION

PGDS is the enzyme responsible for biosynthesis of prostaglandin D_2 (PGD_2) in the central nervous system and is identical to a major CSF protein, β-trace (11–13). PGD_2 had long been considered a minor and biologically inactive prostanoid. In the late 1970s, Hayaishi *et al.* (14) found large amounts of PGDS in the brains of rat and other mammals, including humans. PGD_2 circulates in the ventricular system, subarachnoid space, and extracellular spaces of the brain and interacts with receptors on the ventromedial surface of the rostral basal forebrain to initiate the signal to let the brain sleep (15). PGDS is a member of the lipocalin superfamily composed of various secretory lipophilic ligand-carrier proteins (6, 7). However, its primary role in the brain remains unclear.

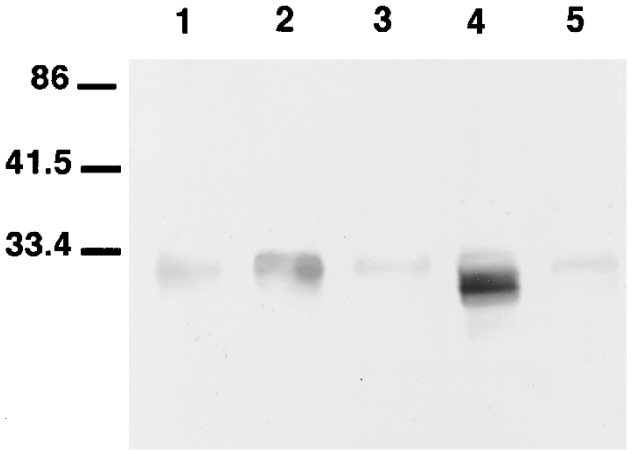

FIGURE 3. An immunoblot probed with anti–prostaglandin D synthase (PGDS) polyclonal antibody. Lanes **1** and **2**, meningeal hemangiopericytomas (HPCs); Lanes **3**, **4**, and **5,** meningiomas. The extracts from meningeal HPCs and meningiomas showed a band (29 kDa) corresponding to PGDS. Prestained molecular weight standards (by Bio-Rad, Hercules, California) are given in kilodaltons on the left.

PGDS is mainly synthesized in the arachnoid cells, the choroid plexus, and oligodendrocytes in the central nervous system (8, 10). Recently PGDS expression in testis and heart has been reported (16, 17). PGDS mRNA is detected by *in situ* hybridization in mouse embryonic mesenchymal cells destined to become arachnoid cells and later in the developing testis (18).

PGDS in the CSF is altered in various brain diseases, including meningitis, multiple sclerosis, subarachnoid hemorrhage, and infarction (19, 20). A recent report revealed that the level of PGDS in CSF increased in various brain tumors (21). It may contribute to regulate the permeability of the meninges (20). PGDS has been considered a specific cell marker of meningiomas. Meningioma cells showed intense immunoreactivity in the perinuclear region, and that was often concentrated within meningocytic whorls and around calcifying psammoma bodies (10). We also examined immunoreactivity for PGDS in three CPPs and seven oligodendrogliomas because choroid plexus and oligodendrocytes are weakly immunopositive for PGDS in the central nervous system. They showed no immunoreactivity for PGDS. Thus, we could strengthen the idea that PGDS has a specificity for meningiomas.

In this study, meningeal HPC showed relatively high frequency (64%) of immunoreactivity to PGDS. Moreover, soft-tissue HPC showed no immunostaining for PGDS except for a single case at the surface of the sacrum, which might be associated with the meninges. Because the histological features so common to meningeal HPC are often encountered in mesenchymal chondrosarcoma, the tumor was also investigated for PGDS immunoreactivity. Because it is rare, only a single case was investigated, and it proved to be immunonegative for PGDS. These findings indicate that meningeal and soft-tissue HPCs are distinctive in view of their PGDS expression. PGDS is widely expressed in the human body; however, the fact that only meningeal HPCs express PGDS means that they may be more closely related to arachnoid cells.

Meningioma and meningeal HPC are currently classified as different entities, but both showed positive immunoreactivity for PGDS. These tumors showed similar perinuclear staining. Yamashima *et al.* (10) presented 100% positive immunoreactivity for PGDS in all meningioma cases, including meningothelial, transitional, fibrous, angiomatous, and atypical meningiomas. In the present study, meningiomas showed a lower frequency of immunopositivity (80%) for PGDS than that observed by Yamashima *et al.* Moreover, the cases that were immunonegative for PGDS consisted of subtypes of fibrous and secretory meningiomas. In transitional meningiomas, menin-

gothelial cells with round to oval nuclei tended to be immunopositive for PGDS. The present study demonstrated a 64% positive rate in meningeal HPCs, which is a lower rate of positivity than that of meningiomas. When a meningeal HPC located in the supratentorial region is compared with meningioma, the difference of PGDS expression is not remarkable (78% *versus* 80%). We examined 12 supratentorial and three infratentorial meningiomas. The meningiomas that showed negative immunoreactivity for PGDS were supratentorial tumors. The positive rates of supratentorial HPCs and supratentorial meningiomas were almost the same (78% *versus* 75%). The common expression of PGDS in meningeal HPC and meningioma may be related to cranial mesenchymal cells. In the central nervous system, primitive mesenchymal cells destined to become arachnoid cells or pericytes exist. Recently, it was reported that multipotent mesenchymal stem cells were isolated from adult bone marrow, and they were induced to differentiate into a variety of mesenchymal tissues (22, 23). *In situ* hybridization studies by Hoffmann *et al.* (18) showed cellular localization of PGDS mRNA during embryonic development of mice. Initially, at 14.5 days postconception, PGDS mRNA was found to be condensed only in the leptomeningeal cells of the brain and spinal cord. Later, at 16.5 days postconception, choroid plexus epithelial cells and single cells within the brain parenchyma were labeled at a significantly lower rate than arachnoid cells. These findings suggest that PGDS plays a more important role in the arachnoid cells than in the choroid plexus and brain parenchyma (oligodendrocytes). It is speculated that if they become neoplastic, PGDS expression may accompany the change. To test this hypothesis, PGDS expression in primitive mesenchymal cells should be investigated.

In conclusion, meningeal HPCs may have a distinct molecular phenotype compared with soft-tissue HPCs, and they are more closely related to the arachnoid cells in origin.

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